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14. ABSTRACT
Clinically significant prostate cancer usually occurs in men who are 65 and older although precursor lesions are known to exist many years prior to cancer diagnosis. Histopathological changes referred to as Prostatic Intraepithelial Neoplasia (PIN) are considered to be the most likely precursor of prostate cancer. The mechanism(s) involved in progression of indolent to active disease remains elusive although a role for age-related increase in oxidative stress has been proposed. There are a variety of reactive oxygen species (ROS) that ultimately cause oxidative stress and any particular oxidant has not been identified as being primarily involved. We rationalized that a combination of antioxidants may be necessary to neutralize the different classes of ROS to prevent the progression of latent precursor foci to active cancer. Therefore we devised a combination of antioxidants with varied antioxidant properties to determine whether such supplementation could prevent the progression of PIN in Noble rats that are stimulated to develop PIN with hormones. Results from this study show for the first time that dietary intervention with a combination of antioxidants caused a highly significant decrease ($p < 0.0004$) in high grade PIN formation compared to animals on control diet possibly through modulation of proliferation/apoptosis induction. These data provide evidence regarding the involvement of oxidants in the progression of precursor lesions and the need to evaluate combinations of antioxidants as prostate cancer preventive agents.

15. SUBJECT TERMS
Oxidative Stress; prostatic intraepithelial neoplasia (PIN); antioxidantsand Prevention

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Introduction:

Although prostate cancer is considered to be a disease of older men, a significant number of relatively young men exhibit the earliest signs of prostate cancer. This suggests that the disease is initiated early and remains latent until some factors trigger it to become malignant. This long latency of prostate cancer progression provides an opportunity for intervention to prevent the initial disease from becoming cancerous. Since treatment options for prostate cancer are very limited for initial stages of the disease and unavailable for metastatic disease, it is imperative that other means to control the disease be vigorously tested to reduce the number of prostate cancer-related deaths in the United States.

Oxidants produced as by-products of cellular metabolism have been implicated in the genesis of prostate cancer. Oxidative stress is caused by an imbalance of cellular endogenous oxidant and antioxidant levels. Laboratory studies using different model systems indicate that oxidative stress markers increase and antioxidant enzyme levels decrease during prostate cancer progression. Oxidative stress generated by dietary fat and androgens has been implicated in prostate cancer. Further epidemiological studies with a variety of antioxidants such as selenium, tocopherols, lycopene, β-carotene etc. have been found to be effective in lowering prostate cancer risk. Although these data suggest the importance of oxidative stress and antioxidants in prostate cancer, they are flawed in that they do not add to our understanding of the nature and amounts of antioxidants that are beneficial. This is extremely important since several classes of oxidants are produced and a single antioxidant cannot quench all the different species of oxidants produced from cellular metabolism. Further, time is an extremely important factor for successful antioxidant prophylaxis. Taken together, the stage of prostate development and the kinds of antioxidants used would play a major role in determining the success

of antioxidant prophylaxis. This proposal is a first step in beginning to understand whether antioxidants can prevent or delay the formation of PIN. Based on evidence presented in the literature, *we hypothesize that a combination of antioxidants can prevent or delay the development of Prostatic Intraepithelial Neoplasia in a T/E$_2$ model of PCA by modulating the level of oxidative stress markers and endogenous antioxidant levels.* To test our hypothesis we propose three specific aims.

1) Determine the ability of antioxidants to prevent or delay the development of Prostatic Intraepithelial Neoplasia (PIN) and relate it to changes in T/E$_2$ in the serum and AR.
2) Determine the levels of oxidative stress markers of DNA, protein and lipids following antioxidant supplementation.
3) Determine the levels and functional ability of endogenous antioxidant components following antioxidant supplementation.

There has been no change in the specific aims proposed.

Key Research Accomplishments:

**We focused solely on completing the tasks as proposed in the grant application.**

The above mentioned grant was awarded to AMC Cancer Research Center, Denver, CO in March 2003. The PI's laboratory relocated to the University of Texas Health Science Center, San Antonio, TX in July 2005. PI's laboratory became functional only in January of 2006. In addition the award was transferred to UTHSCSA in February 2006. Subsequently PI hired new staff and work was initiated. **This work is currently ongoing and the results are encouraging. We have presented some of this data as an abstract at the AACR meeting in 2006 and the manuscript describing this work is under preparation (draft enclosed).** Due to this relocation and time involved in setting up the laboratory at the new location experiments to accomplish the proposed tasks are still ongoing. **In order to successfully complete the proposed work we have requested no cost extension for an additional year until the end of March 2008.**

The age-related nature of prostate cancer combined with increased life expectancy of men in the developed world is poised to make prostate cancer a major health crisis soon. PIN is the abnormal proliferation within prostatic ducts, ductules, and large acini without stromal invasion. In the United States an estimated 115,000 new cases of high grade PIN are diagnosed each year (1-2). In a study that included young men in their twenties and thirties the frequency of PIN was approximately 9 and 20% respectively (3). Most PIN foci in young men are low grade and increase to high grade and volume with increasing age (4). Therefore understanding the characteristics of PIN and its progression will allow the development of agents that can prevent or delay the progression of precursor lesions to cancer. This would decrease health care related costs and provide a better quality of life for elderly men. A shift in oxidant/reductant balance in cells towards a prooxidant state is associated with aging and has been suggested as an important mechanism in prostate cancer. Decreased expression of endogenous antioxidant enzymes is associated with PIN and prostate cancer (5). Antioxidants such as vitamin E, selenium and lycopene have been shown to inhibit growth of prostate cancer cells in culture (6-10). Recently administration of vitamin E, selenium, and lycopene was shown to prevent prostate cancer development in the Lady transgenic mouse model (10). A large randomized placebo controlled study to test the prevention of prostate cancer using selenium and vitamin E is ongoing in humans. While these studies suggest the importance of antioxidants in prostate cancer they do not address the role of oxidants in the progression of indolent disease to clinically significant cancer. The current study differs from published literature in that combinations of antioxidants that quench different oxidants have been used to target progression of PIN as an endpoint in an animal model. Noble rats develop a high incidence of prostate carcinomas in response to hormone stimulation that is preceded by multiple dysplastic lesions that resemble human PIN in origin, morphology and biological characteristics (11-12). Further tumors occur in dorsolateral prostate which is relevant to the site of origin of PIN and carcinoma in humans (13-14). Therefore this model provides the opportunity to address the prophylactic role of antioxidants in the progression of latent form of prostate cancer to overt disease.

Antioxidant	Low dose	High dose
Ascorbic acid	3	6
Vitamin E acetate	6	15
Riboflavin	0.3	0.6
Beta carotene	0.85	1.9
Selenium	0.065	0.125
Lycopene	0.5	1
Lutein	0.5	1
Alpha Lipoic acid	1.5	3
Grape seed extract	5	10
Co-enzyme Q10	5	10

Table 1 Composition and dose of antioxidants used in the study. Ascorbic acid is a potent antioxidant that interacts synergistically with Lipoic acid to destroy many types of free radicals. Vitamin E is fat soluble and works synergistically with ascorbic acid, selenium and co-enzyme Q_{10}. Riboflavin is a water-soluble vitamin, a cofactor necessary for efficient function of glutathione reductase. β-carotene is a precursor of vitamin A and it exhibits very strong antioxidant properties in combination with vitamins C, E and selenium. Selenium is an essential component of glutathione peroxidase and is important for the biosynthesis of co-enzyme Q_{10}. Lycopene and lutein are fat soluble carotenoids that work synergistically and possess very high antioxidant activity. Lipoic acid not only destroys radicals but also recycles glutathione and co-enzyme Q_{10} back to the reduced state. Grape seed extract is rich in proanthocyanidins and anthocyanins that destroy different radicals as well as potentiate other antioxidants such as glutathione. Co-enzyme Q_{10} is a lipid-soluble, natural antioxidant produced by cells. It plays an important role in breaking the free-radical chain in membranes. The doses of antioxidants in the diet were based on daily human consumption of vitamin supplements. Doses are in g/kg diet.
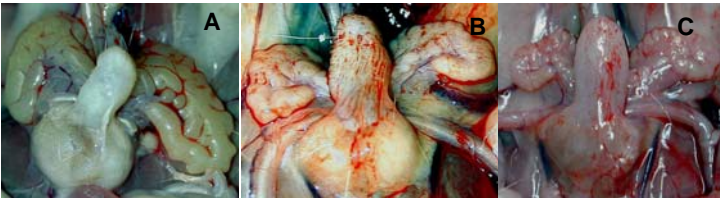
Figure 1 Prostate-seminal vesicles complex (PSVC) from Noble rats. PSVC at the termination of the experiment did not show any gross abnormalities. A. animals that were on control diet and not stimulated with hormones; B. animals on control diet stimulated with testosterone and estradiol; C. animals on low dose of antioxidant supplemented diet and stimulated with hormones.
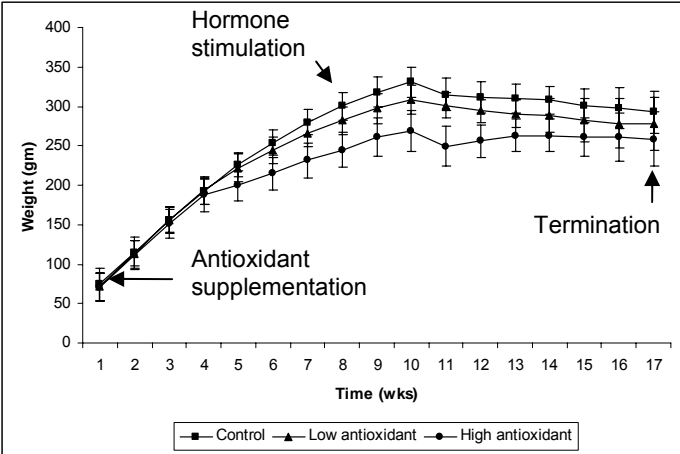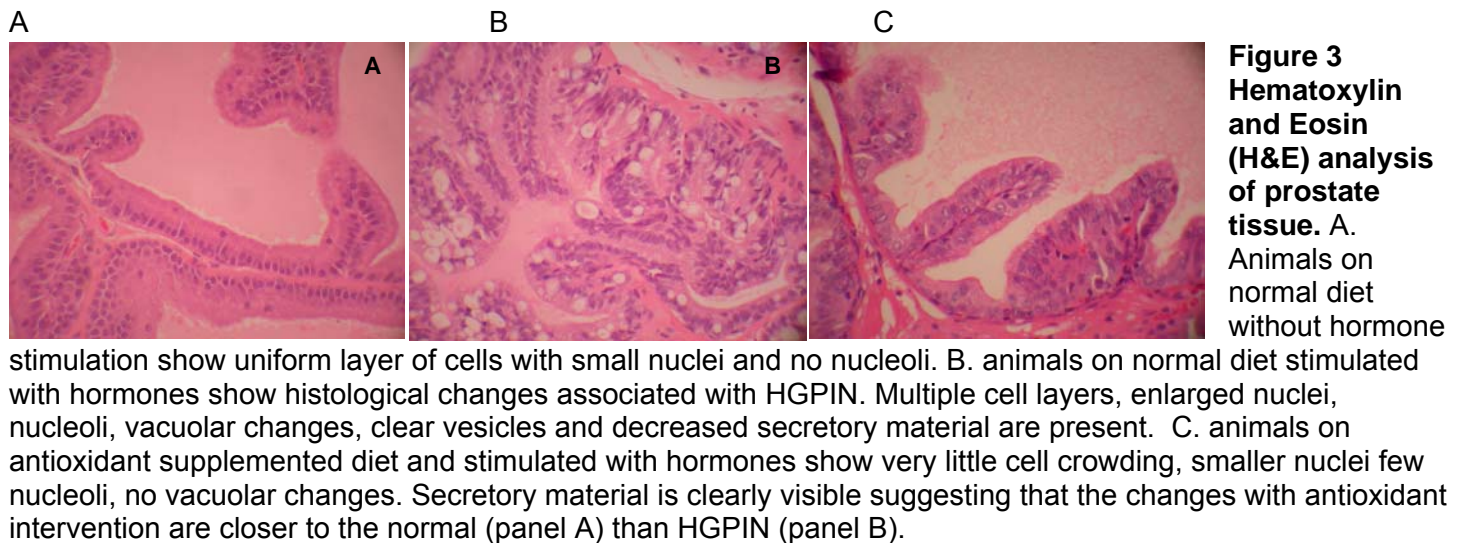

Figure 2 Mean body weight of Noble rats. Mean body weight in each experimental group $\pm$ sd as a function of time is shown in weeks. Dietary antioxidant supplementation was started in the intervention group when animals were 6 weeks old and it lasted for 7 weeks. Animals were moved back to normal diet and then stimulated with hormones and the stimulation lasted for 9 weeks. Termination indicates body weight at the time of termination of the experiment.

Table 2 Body weight changes in response to antioxidant intervention. ANOVA results show that the group that did not receive antioxidant supplemented diet gained significantly more weight in the first 66 days (9 weeks; when all the animals were alive) of the experiment than the combined groups that received the antioxidant supplemented diet. There was also a significant dose-response effect with the low dose group gaining more weight than the high dose group. Food intake data showed that animals that were on the control diet consumed more food than the animals on the antioxidant diet in the first 66 days of the experiment (data

not shown). It could either be due to the taste of the food or that smaller quantities were more filling. However the changes in body weight as well as food intake dissipated to no-significance by day 112 (at the termination of the experiment). Rank transformed ANOVA results (not shown) were entirely concordant with the results presented in Table 2 i.e. both had p <0.025 or both had p >0.025. One animal in the high dose antioxidant group dies after stimulation with hormones. It was not an-antioxidant-intervention related death. Further there was no significant difference in the weight of the genitourinary (GU) apparatus between control and antioxidant supplemented groups at the time of sacrifice.

Figure 3 Hematoxylin and Eosin (H&E) analysis of prostate tissue. A. Animals on normal diet without hormone stimulation show uniform layer of cells with small nuclei and no nucleoli. B. animals on normal diet stimulated with hormones show histological changes associated with HGPIN. Multiple cell layers, enlarged nuclei, nucleoli, vacuolar changes, clear vesicles and decreased secretory material are present. C. animals on antioxidant supplemented diet and stimulated with hormones show very little cell crowding, smaller nuclei few nucleoli, no vacuolar changes. Secretory material is clearly visible suggesting that the changes with antioxidant intervention are closer to the normal (panel A) than HGPIN (panel B).

Table 2. Means (95% confidence intervals) for change in weight from initial weighing and end of experiment GU weight by experimental groups

Measure (g)	No special Diet	p†	Low dose	p‡	High dose
N	10		10		9-10§
Δweight to day 66	246 (225, 267)	**0.0042**	234 (219, 249)	**<0.0001**	182 (167, 197)
Δweight to day 112	218 (199, 238)	0.082§	206 (187, 225)	0.21	188 (168, 209)
GU weight on day 112*	1.51 (0.99, 2.29)	0.66	1.08 (0.68, 1.72)	0.16	1.72 (1.06, 2.79)

† Comparing No special diet to the special diet groups combined.
‡ Comparing low dose to high dose special diet groups.
* Log-transformed for analysis and back-transformed for presentation.
§ day 66 N = 10; day 112 N = 9
ξ p= 0.0367 from rank-transformed ANOVA

Table 3 Fisher's exact test results of PIN formation. Data presented in table 3 show that there was a highly significant (p= 0.004) difference in HGPIN formation between animals that were on the control diet compared with those on the antioxidant supplemented diet. A p-value = 1 may be interpreted as indicating the observed results were as close as possible to the expected results given the null hypothesis of no association. These data suggest that the group that did not receive the special diet had a significantly higher proportion of animals

progressing to HGPIN. In contrast higher proportion of animals on antioxidant supplemented diet did not progress beyond low grade PIN. Examination of the food consumption data (not shown) leads us to suggest that animals that developed HGPIN in the antioxidant groups may have been those that did eat enough food and therefore did not receive the dose of antioxidants necessary to prevent the progression of LGPIN to HGPIN.

The histopathological data shown here was analyzed by one GU pathologist (Dr Dean A Troyer). Two additional pathologists are currently analyzing the histopathology slides in a blinded fashion (grading is described in the methods section). While 1 animal in each group did not develop PIN with hormone stimulation 80% of animals on control diet had HGPIN at the time of sacrifice. In contrast 90% of the animals on antioxidant supplemented diet had near normal to LGPIN and only 10% had progressed to HGPIN. This clearly demonstrates that antioxidant intervention inhibited HGPIN formation by about 90% in Noble rats.

Table 3. N (%) by experimental groups and results from Fisher's exact tests.

|	Control			Antioxidant diet	
Measure (g)	Diet	p†	Low dose	p‡	High dose
Total	10		10		10
Died < end of experiment	0 (0%)	1.00	0 (0%)	1.00	1(10%)*
No PIN	1 (10%)	1.00	1 (10%)	1.00	1 (11%)
Low grade PIN	1 (10%)	§	8 (80%)	§	7 (78%)
High Grade PIN	8 (80%)	**0.0004**	1 (10%)	1.00	1 (11%)

† Comparing no special diet to the special diet groups combined.
‡ Comparing low dose to high dose special diet groups.
* excluded from other analyses.
§ Comparison not made as it would be either not meaningful or redundant to rows immediately above or below.

To determine the status of proliferation of prostatic epithelium cells we stained prostate tissues from animals for the proliferation marker Ki67. Animals on antioxidant-supplemented diet stimulated with hormones showed more than 50% decrease in proliferation compared with their counterparts that received the control diet. The level of proliferation in the antioxidant group was similar to the group that did not receive hormone stimulation. There was no dose-response in the expression of Ki67 between the low and high dose groups. TUNEL staining was used to determine apoptosis induction. Tissue from animals that were not stimulated with hormones showed no staining similar to the hormone-stimulated animals on control diet. However animals on antioxidant-stimulated diet that was subject to hormone stimulation had a high percentage of cells that showed positive TUNEL staining. There was no dose-response increase in the TUNEL positive cells with antioxidants.
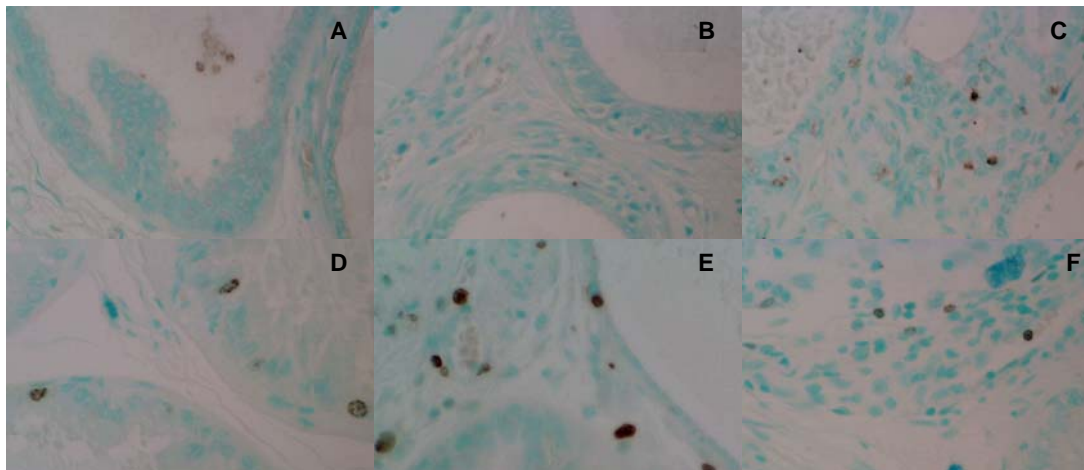
Figure 4

Immunohistochemistry of proliferation marker Ki67 and apoptosis in prostate tissues. TUNEL staining of prostate tissues obtained from animals in the different groups is shown in A-C. A. Animals on control diet not stimulated with hormones; B. animals on control diet stimulated with hormones and C. animals on low antioxidant dose stimulated with hormones. Immunohistochemistry with the proliferation marker Ki67 is shown in panels D-F. Proliferation in animals on control diet not stimulated with hormones is shown in panel D. Increased proliferation in tissues obtained from animals on control diet stimulated with hormones is shown in panel E. Decreased proliferation in those animals treated with hormones but on low antioxidant dose is shown in panel F.

Methods

Animal manipulation: Noble rats were purchased from Charles River Laboratories (Wilmington, MA). Animal experiments were conducted in accordance with approved protocols by the institutional animal care committee. Animals were housed in groups of 4 under a 12 hour light-dark cycle and a temperature of 23 ± 2^oC with access to food and water. At 6 weeks of age animals were randomized into 2 groups of 10 animals each for antioxidant intervention. One group of 10 animals received low dose of antioxidant while the other group received high dose of antioxidants. Control and special diet (AIN-93G supplemented with antioxidants shown in the table 1) was obtained from Dytes Inc., (Bethlehem, PA). 20 animals received AIN-93G diet until the end of the study. Food cups were weighed before and after feeding to determine the amount of food and antioxidant consumed. All animals were weighed weekly and observed daily for signs of illness. Antioxidant intervention lasted for 7 weeks.

The intervention group animals were put back on control diet prior to stimulation with hormones so that antioxidants did not modulate hormone level and or activity. All animals except group 1 were treated with testosterone and estradiol. Slow release pellets containing 240 mg testosterone propionate and 25 mg 17 - estradiol benzoate (Innovative Research America, FL) were implanted *sc* into the flanks of the animals. Control animals received placebo pellets. Hormone stimulation lasted for 9 weeks.

Animals were sacrificed by CO_2 asphyxiation followed by cervical dislocation. The abdominal cavity was opened and all the organs were examined for gross changes. Prostate was dissected from the rest of the genitourinary organs, weighed, cut longitudinally along the urethra, and fixed in 10% buffered formalin.

Histopathology of prostate lesions: Serial sections of prostate tissue were stained with H&E. PIN was diagnosed according to the criteria suggested by Leav et al (11). According to these criteria PIN in Noble rats is recognized by the presence of multiple layers of dysplastic epithelial cells that form alveolar or papillary structures, increased nuclear and nucleolar size, variability in shape and stainability. Prostate tissues exhibiting the presence of variable nuclear enlargement and irregular cell spacing with some nuclear stratification and crowding were denoted with + and graded as low grade PIN (LGPIN). Cells that had additional nuclear enlargement, fine nuclear chromatin pattern, with prominent nucleoli were denoted +++ and graded as high grade PIN (HGPIN).

Statistical analysis: Changes in weight from the initial weighing on day 1 were calculated at two time points: day 66 (last weighing when all animals were alive) and day 112 (final weighing prior to termination). Analysis of variance (ANOVA) and Fisher's exact tests were performed using SAS version 9.1 to assess the effect of either special diet compared to the group that received no special diet and the dose-response effect comparing the high-dose group to the low-dose group. Since two comparisons were performed for each measure we considered $p < 0.025$ significant. Measures were log transformed for analysis and back-transformed for presentation if their log-transformed distribution appeared to be more normally distributed than their untransformed distribution. Rank transformed ANOVAs were also performed for each continuous measure.

Reportable outcomes: The outcome of the study is that antioxidant supplementation significantly reduced the development of high grade PIN. Part of this work was presented as an abstract at the AACR meeting in

Washington DC (2006). We are in the process of submitting this work for publication (confirming the histopathological analysis through two independent GU pathologists).

Conclusions: Primary management of prostate cancer for a majority of patients consists of radical surgery or radiation therapy. Although this is adequate for disease control in some patients a significant number of patients relapse and ultimately develop metastatic disease. There are limited treatment options for patients who have undergone primary therapy with curative intent. Early initiation of hormonal ablation is associated with significant morbidity and effect on quality of life including hot flashes, loss of libido, decreased muscle mass, and osteoporosis with long term use. Since PIN precedes prostate cancer delaying the progression of PIN or reversing HGPIN to LGPIN serves as an excellent mechanism to ensure quality of life for elderly men. Several lines of evidence suggest a beneficial role for vitamin consumption against prostate cancer. In this context Meyer and colleagues have shown that supplementation with nutritional doses of vitamin C, vitamin E, β-carotene, selenium and zinc daily for 8 years significantly reduced the rate of prostate cancer development in men with normal PSA (< 3ng/ml; 15). The α-tocopherol, β-carotene (ATBC) cancer prevention trial in Finland found that consumption of vitamin E reduced clinical prostate carcinoma by 32% and prostate cancer mortality by 41% and no effect of vitamin E on latent prostate cancer (16). The double-blinded selenium chemoprevention trial by Clark and colleagues originally directed towards high-risk skin cancer patients found that selenium reduced prostate carcinoma risk significantly (17-18). While these studies suggest a role for antioxidant vitamin supplementation in the development of prostate carcinoma they do not shed any light regarding their effectiveness in preventing the progression of early PIN lesions towards clinically significant prostate cancer.

In this report we have shown that by supplementing the diet of Noble rats prior to stimulating with hormones LGPIN does not progress to HGPIN. At this time we do not know whether this supplementation has resulted in delaying the progression of LGPIN to HGPIN or whether HGPIN formation has been completely suppressed in these animals. We stopped dietary antioxidant supplementation before inducing the animals with hormones to ensure that both the control and antioxidant groups received hormone stimulation under the same conditions. Yet a vast majority of the animals in the special diet group did not develop HGPIN suggesting that the antioxidants modified the prostate environment in a way to prevent the progression of LGPIN to HGPIN upon hormone stimulation. Our results also suggest that antioxidant intervention enabled the environment not only to remove damaged cells through induction of apoptosis but also suppressed hormone-induced proliferation of prostatic epithelial cells. Since the animals were stimulated with hormones under the same conditions we were able to separate the effect of antioxidants on hormone levels from the effect of the antioxidants on PIN development. The levels of testosterone or the ratio of testosterone to estradiol at the end of the study was not significantly different between animals on control vs. special diet (data not shown). None of the animals in any group were found to have gross abnormalities in kidney, bladder, seminal vesicle, prostate and liver. The data from this study clearly demonstrate the importance of an antioxidant combination in preventing the progression of precursor LGPIN to HGPIN in the noble rat model.

Although this study was not designed to examine the ability of antioxidants to extend the life of the animals stimulated to develop prostate cancer, it is an important discovery as a prophylactic for hundreds of thousands of men on 'watchful waiting' for their latent disease to progress to full blown cancer. Alternatively this antioxidant combination may be a useful adjuvant for men who have undergone androgen deprivation therapy as well as radiation therapy. Since dysplastic prostatic epithelium is considered to be hormone-dependent, androgen deprivation has been found to decrease high grade PIN by 50% (19-20). However it is also known that neoplastic cells that arise subsequently are not responsive to hormone deprivation. Since antioxidant intervention caused a significant decrease in HGPIN formation our results suggest that it may work through androgen-independent mechanism and may be useful in post-androgen deprivation therapy. It may be useful even for men who have received radiation therapy that does not successfully remove all HGPIN foci. A report from Memorial Sloan Kettering found PIN in 8.8% of biopsies after a course of 3-dimensional external beam conformal radiation therapy (21). Currently there is neither a routine treatment nor prevention format for HGPIN. This antioxidant combination holds promise to fill this void.

References:

1.Greenlee, R, Hill-Harmon, M, Murray, T. et al 2001 Cancer statistics CA Cancer J Clin 51: 15-36

2. Steiner, MS, Raghow, S, Neubauer, BL, 2001 Selective estrogen receptor modulators for the chemoprevention of prostate cancer. Urology: 57: 68-72

3. Sakr, WA, Haas, GP, Cassin, BF et al 1993 The frequency of carcinoma and intraepithelial neoplasia of the prostate in young male patients J Urol 150: 379-85

4. Qian, J, Wollan, P, Bostwick, DG 1997 The extent and multicentricity of high-grade prostatic intraepithelial neoplasia in clinically localized prostatic adenocarcinoma. Human Pathol 28: 143-48

5. Bostwick, DG, Alexander, EE, Singh, R et al 2000 Antioxidant enzyme expression and reactive oxygen species damage in prostatic intraepithelial neoplasia and cancer. Cancer 89: 123-34

6 Menter, D. G., Sabichi, A. L. and Lippman, S. M. 2000. Selenium effects on prostate cell growth. Cancer Epidemiology Biomarkers Prev. 9: 1171-1182.

7. Venkateswaran, V., Fleshner, N. E., and Klotz, L. H. 2004. Synergistic effect of vitamin E and selenium in human prostate cancer cell lines. Prostate cancer Prostatic Dis. 7: 54-56.

8. Horvath, P. M. and Ip, C. 1983. Synergistic effect of Vitamin E and selenium in the chemoprevention of mammary carcinogenesis in rats. Cancer Res. 43: 5335-5341.

9. Ip, C., White, G. 1987. Mammary cancer chemoprevention by inorganic and organic selenium: single agent treatment or in combination with vitamin E and their effects on in vitro immune functions. Carcinogenesis. 8: 1763-1776.

10. Venkateswaran, V., Fleshner, N. E., Sugar, L. M. and Klotz, L. H. 2004. Antioxidants block prostate cancer in Lady Transgenic mice. Cancer Research. 64: 5891-5896.

11. Leav, I, Ho, S-M, Ofner, P et al 1988 Biochemical alterations in sex hormone-induced hyperplasia and dysplasia of the dorsolateral prostates of noble rats. J Natl. Cancer Inst. 80: 1045-52.

12. Leav, I, Merk, FB, Kwan, PW-L, Ho, S-M. 1989 Androgen-supported estrogen-enhanced epithelial proliferation in the prostates of intact Noble rats. J Natl. Cancer Inst. 15: 23-40

13. Noble RL 1982 Prostate carcinoma of the Nb rat in relation to hormones. Int Rev Exp Pathol 23: 113-59

14. Tam, NN, Chung, SS, Lee, DT et al 2000 Aberrant expression of hepatocyte growth factor and its receptor, c-Met, during sex hormone induced prostatic carcinogenesis in the Noble rat. Carcinogenesis 21: 2138-91

15. Meyer, F, Galan, P, Douville, P. 2005. Antioxidant vitamin and mineral supplementation and prostate cancer prevention in the SU.VI.MAX trial. Int J Cancer. 116: 182-186

16. Hennekens, CH, Buring JE, Manson, JE et al. 1996 Lack of effect of long-term supplementation with beta carotene on the incidence of malignant neoplasms and cardiovascular disease. N Engl. J Med. 334: 1145-1149.

17. Clark LC, Combs GF Jr., Turnbull BW et al 1996 Effects of selenium supplementation for cancer prevention in patients with carcinoma of the skin. A randomized controlled trial. Nutritional Prevention of Cancer Study Group. JAMA 276: 1957-63

18. Brooks JD, Metter EJ, Chan DW. et al 2001 Plasma selenium level before diagnosis and the risk of prostate cancer development. J Urol. 166: 2034-2038.

19. Balaji, KC, Rabbani, F, Tsai, H et al 1999 Effect of neoadjuvant hormonal therapy on prostatic intraepithelial neoplasia and its prognostic significance. J Urol. 162: 753-57

20. Ferguson, J, Zincke, H, Ellison, E et al 1994 Decrease of prostatic intraepithelial neoplasia following androgen deprivation therapy in patients with stage T3 carcinoma treated by radical prostatectomy. Urology 44: 91-95

21. Gaudin, PB, Zelefsky, MJ, Leibel, SA et al 1999 Histopathologic effects of three-dimensional conformal external beam radiation therapy on benign and malignant prostate tissues. Am J Surg. Pathol. 23: 1021-31

Manuscripts under preparation:

- Ghosh, R., Garcia, G. E., Arevalo, N., I-Tien Yeh, Troyer, D. A. Chan, D. E and Kumar, A. P. Antioxidant combination blocks progression of prostatic intraepithelial neoplasia.
- Ghosh, R., Garcia, G. E., Arevalo, N., Smith, M., M Scott Lucia and Kumar, A. P. Hormone induced inflammatory changes in the ventral prostate of Nobel rats.